PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
W 3435-004		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE2005/000220	17-02-2005	17-02-2004
International Patent Classification (IPC) or national classification and IPC		
See Supplemental Box		
Applicant		
Synbiotics AB et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 10 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 1 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.09.2005	24-02-2006
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Terese Sandström/EÖ Telephone No. +46 8 782 25 00

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Cover sheet

INTERNATIONAL PATENT CLASSIFICATION (IPC):

A61K 35/74 (2006.01)
A61P 29/00 (2006.01)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2005/000220

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____ ,
which is the language of a translation furnished for the purposes of:

☐ international search (Rules 12.3(a) and 23.1(b))

☐ publication of the international application (Rule 12.4(a))

☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-18 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 1 (claims 1-12) received by this Authority on 20-02-2006

pages* _____ received by this Authority on _____

☐ the drawings:

pages _____ as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority document discloses the use of a composition comprising 4 specified lactic acid bacteria for preventing or treating stress-induced inflammatory disorders whereas the present application relates to the use of at least two of the specified lactic acid bacteria for preventing or treating stress-induced inflammatory disorders. Thus, for subject matter considering a composition of two or three of the specified lactic acid bacteria the claimed priority is not valid. However, since the present claims are all restricted to compositions comprising all four specified lactic acid bacteria, documents 'Liu Q. et al., "Synbiotic Modulation of Gut Flora: Effect on Minimal Hepatic Encephalopathy in Patients With Cirrhosis", Hepatology, April 2004, Vol. 39, pages 1441-1449', 'Möller A. et al., "Changes in cytokine concentration during enrichment of early nutrition with lactic acid bacterium (Synbiotic 2000) after major abdominal surgery", Critical Care, March 2004, Vol.8, Suppl. 1, P273' and WO 2004/103083, all disclosing compositions comprising the four lactic acid bacteria, are not included in this report.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 12

because:

☒ the said international application, or the said claims Nos. 12 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☐ no international search report has been established for said claims Nos. _____

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in the Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-11	YES
	Claims		NO
Inventive step (IS)	Claims	1-11	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The present claims relate to the use of a composition comprising the four lactic acid bacteria Pediococcus pentosecus 5-33:3, Leuconostoc mesenteroides 32-77:1, L. paracasei subsp. paracasei 19 and L. plantarum 2362, wherein the bacterial strains are in an amount of at least 10^{11} CFU/ml of each of the bacteria, in combination with at least four different fibres for the manufacturing of a formulation for the prevention of a stress-induced inflammatory disorder.

Documents cited in the International Search Report:
D1: Colucci G. et al., "Prevention of Postoperative Adhesions after Abdominal Aortic Surgery", Eur. Surg. Res., 2003, Vol. 35, page 265; P25
D2: Bengmark S., "Use of some pre-, pro- and synbiotics in critically ill patients", Best Practice & Research Clinical Gastroenterology, 2003, Vol. 17, No. 5, pages 833-848
D3: Bengmark S. "Modulation by enteral nutrition of the acute phase response and immune functions", Nutr. Hosp., 2003, Vol. 18, No. 1, pages 1-5
D4: Bengmark S., "Synbiotic Control of Inflammation and Infection in Transplantation", Transplantation Reviews, January 2004, Vol. 18, No. 1, pages 38-53

D1 discloses the administration of "Synbiotic 2000" to mice undergoing surgery. It was demonstrated that the use of "Synbiotic 2000" reduced inflammation response in those mice. "Synbiotic 2000" consists of the four lactic acid bacteria Pediococcus pentosecus 5-33:3, Leuconostoc mesenteroides 32-77:1, L. paracasei subsp. paracasei 19 and L. plantarum 2362 in combination with the four fermentable fibres beta-glucan, inulin, pectin and resistant starch (see e.g. D2 for the specific fibres). The composition in D1 includes 10^{8} CFU of each of the lactic acid bacteria.

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

D2 discloses the use of "Synbiotic 2000" for the treatment of chronic distal colitis. Patients with chronic distal colitis treated with "Synbiotic 2000", administered twice-daily enemas, showed e.g. significant reductions in diarrhoea scores as well as visible blood in the stools. (Page 844, paragraph 2.) The composition used included 10^{10} CFU of each of the four lactic acid bacteria.

D3 suggests the use of "Synbiotic 2000" to modulate the acute phase response and limit induced superinflammation. (Page 4, column 1, last paragraph -column 2, paragraph 1.) D3 does not disclose the amount of bacterial strains in the composition. However, looking at the state of the art, "Synbiotic 2000" seems to refer to a composition comprising the 4 bacterial strains in amounts of 10^{8} or 10^{10} CFU/ml.

D4 suggests the use of synbiotic in general for reducing inflammation of the liver. D4 discloses some synbiotic compositions, one composition comprising 1 lactic acid bacteria in combination with one fibre and two compositions comprising 4 lactic acid bacteria in combination with four fibres ("Synbiotic 2000" and "Synbiotic FORTE"). "Synbiotic FORTE" differs from "Synbiotic 2000" since it comprises 10^{11} of each of the four lactic acid bacteria. The composition comprising one lactic acid bacteria in combination with one fibre was shown to treat pancreatitis as well as to reduce inflammation in the liver. (Page 48, column 1, paragraph 1; page 49, column 1, last 4 lines.)

The use as claimed in the present claims is not disclosed by any of the documents D1-D4. None of the documents describes the use of the four specific bacterial strains as defined in the claims in an amount of at least 10^{11} CFU/ml of each of the bacteria and at least four fibres for the manufacture of a formulation for the prevention, i.e. not the treatment, of a stress-induced inflammatory disorder. Hence, the subject matter claimed in claims 1-11 is novel.

None of these documents are focused on solving the problem of preventing stress-induced inflammatory disorders, even though the inflammation in D1 might be considered to be a stress induced inflammatory disorder.

D4, which disclosed the composition "Synbiotic FORTE", is considered to be one document disclosing the closest prior art.

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

Since "Synbiotic FORTE", a composition comprising 10^{11} CFU/ml of each of the lactic acid bacteria, is one of the synbiotic compositions reviewed in the article, it might seem close to hand for a person skilled in the art, wishing to reduce inflammations in general or even stress-induced inflammatory disorders, to use this synbiotic composition. However, a person skilled in the art faced with the problem of preventing a stress-induced inflammatory disorder coming across D4 would assume that to prevent a disease instead of treating/curing a disease could easily be done by the use of solely a combination of oat and L. plantarum (the composition used in D4 comprising one lactic acid bacteria and one fibre) and would not try to prepare a mixture of four different specific bacterial strains and four fibres in the large amount of 10^{11} CFU/ml (as in the case on the "synbiotic FORTE" composition). It is less expensive and less complicated to use a less complicated composition.

The main difference between the subject matter claimed in claims 1-11 and the prior art stated in D1-D4 is the high amount of the four bacterial strains.

According to the response of the applicant, the inventors have surprisingly found that the use of such high amounts of the four bacterial strains in combination with four fibres gives rise to the following activities within the person to which the compositions is administrated, making it suitable for the claimed use:

The expression of the heat shock proteins increases
The expression of the nuclear factor (NF) kappa B decreases
TNF-alfa, Interleukin (IL)-6 and other markers of inflammation decreases
Caspase-3 cell and tissue expression decreases
Human leukocyte antigen (HLA)-DR expression improves
The NF-kB expression is reduced
COX 2 expression is reduced
The iNOS expression is reduced
The PAI-1 activity if reduced
The tissue infiltration of neutrophils is prevented
Tissue destruction is prevented
Gut for a is restored

This difference is not predictable to a person skilled in the art in view of the documents cited above.

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

Hence it is not considered obvious for a person skilled in the art to use the four specific bacterial strains as defined in the claims in an amount of at least 10^{11} CFU/ml of each of the bacteria and at least four fibres for the manufacture of a formulation for the prevention, i.e. not the treatment, of a stress-induced inflammatory disorder.

To summaries, the subject matter claimed in claims 1-11 is novel and is considered to involve an inventive step. The subject matter claimed in claims 1-11 is considered to be industrially applicable.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO2004103083 A1	12.12.2004	18.05.2004	22.05.2003

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

PCT / SE 2005 / 0 0 0 2 2 0
2 0 -02- 2006

CLAIMS

1. Use of *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp paracasei F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606) wherein the bacterial strains are in an amount of at least 10^{11} CFU/ml of each of the bacteria and at least four different fibres for the manufacturing of a formulation for the prevention of a stress-induced inflammatory disorder.
2. Use according to claim 1, wherein the stress-induced inflammatory disorder is determined as an increase in neutrophils, cytokines, myeloperoxidase and/or accumulation of the oxidation-related malonedealdehyde.
3. Use according to any of proceeding claims, wherein the inflammatory disorder is lung inflammation, urinary inflammation, vaginal inflammation, bowel inflammation, stomach inflammation, liver inflammation, muscle inflammation, inflammation of endocrine and reproductive organs, and brain inflammation.
4. Use according to claim 3, wherein the fibre is selected from the group consisting of beta-glucan, inulin, pectin, resistant starch, cellulose, hemicellulose, arabinoxylans, arabinogalactans, polyfructose, inulin, oligofructans, galacto-oligosacharides, gums, mucilages, pectins, dextrins, maltodextrins, potato dextrins, synthesised carbohydrates, polydextrose, methylcellulose and hydroxypropylmethylcellulose.
5. Use according to claim 4, wherein the four fibres are inulin, beta-glucan, pectin and resistant starch.
6. Use according to claim 5, wherein the fibres are present in an amount of 2.5 g of each fibre.
7. Use according to claim 4, wherein the fibre is selected from lignin substances from plants selected from the group comprising waxes, cutin, phytate, saponin, suberin and tannins.
8. Use according to any of proceeding claims, wherein the formulation further comprises at least one antioxidant, vitamin, mineral, amino acid, peptide or protein.
9. Use according to any of proceeding claims, wherein the formulation further comprises glutamine, or a synthetic version thereof.
10. Use according to any of proceeding claims, wherein the formulation further comprises one or more therapeutic agents.
11. Use according to any of preceding claims, wherein the formulation is solid or liquid, such as tablet, gel or spray.
12. Use of the formulation according to any of proceeding claims for the prevention of a mammal suffering from a stress-induced inflammatory disorder, such as an animal or human being.